RESEARCH ARTICLE

Reversible Antifertility Effect of *Cassia tora* Linn in Male Rats

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ABSTRACT
Background: Plant *Cassia tora* has been used in traditional and modern medicines for different pharmacological activities. The present investigation has been observed and evaluated the effects of *C. tora* on the reproductive functions of male rats in search a safe, orally effective and reversible fertility regulating agent.
Methods: Fifty percent ethanolic extract of *C. tora* was prepared and administered orally in male Wistar rats at the doses of 50, 100 and 200 mg/kg.b.wt./rat/day dose levels respectively, for a period of 60 days and some of the treated rats were kept 30 days for recovery of fertility to assess reversibility effects. Hematological indices, serum clinical investigations were also performed to assess toxic effects, if any caused in rats by treatment. Proteins, cholesterol, glycogen, ascorbic acid, sialic acid and fructose level were analyzed in rats. Serum FSH, LH and Testosterone levels were measured. Rats were castrated to evaluate effects on reproductive functions of hormones and mode of action of the *C. tora* treatment. For histopathological observations tissues were fixed in Bouin's fluid, dehydrated, sectioned and stained with Hematoxylin and Eosin.
Results: Treatment of *C. tora* significantly reduced the weights of testes and accessory sex organs. Sperm density and motility were declined high significantly. Levels of Testosterone and FSH hormone were significantly decreased in rats. The protein, sialic acid, fructose, ascorbic acid and glycogen contents of reproductive accessory sex organs were decreased significantly. Germinal epithelium of testes degenerated and number of spermatocytes, spermatids and spermatozoa in the lumen of seminiferous tubules reduced.
Conclusion: The decreased testes and accessory sex organ weights, sperm motility, density and testosterone level in rats might be due to androgen suppression effects of *C. tora* treatment cause inhibition of spermatogenesis resulted reduction of fertility in treated male rats.

Key-words- *Cassia tora,* Contraception, Fertility, Sperm motility, Sperm density, Male rat

INTRODUCTION
The population explosion creates immense pressure on our natural and non-renewable resources, leading to social economic imbalances and political tension [1,2]. Fertility regulation has therefore, become a major global concern [3-5]. Although, there are several types of contraceptive methods are available for fertility control in male and females, but all of these were found one or more side effects. Survey of literature enumerate that the use of plant products as anti-fertility agent cause minimal or no side effects as compared to current available conventional contraceptive methods especially oral. The literature reveals that the plants and their products were used for fertility regulation since hazards of uncontrolled population were visualized [6-9].

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World Health Organization have already been taken significant steps to carry out research aimed at finding new and effective anti-fertility agents from traditional medicinal plants [10,11].
The results obtained are encouraging and thus hoped that in the near future an easily administered and reversible oral contraceptive of plant origin would be available to common people. The present investigation is planned to evaluate contraceptive efficacy of *C. tora* fruits products to search a cheap, orally effective and reversible contraceptive from indigenous medicinal plants with emphasis on mode of action and side effects in rats with the objective to develop a cheap, safe, easily administrable, orally effective and reversible male fertility regulating agents from traditional medicinal plants.

MATERIALS AND METHODS
The Plant- The plant *C. tora* belongs to family- Fabaceae and also known as *C. obstusifolia*, Foetid cassia, Sickle senna, Wild senna, Charota, Chakvat, Chakvat senna tora etc [12] have selected for the present study on the basis of literature survey of

ethanomedicinal properties. The fruits of the plant were collected in the months of September and October of 2010 and study was carried out at Department of Zoology, University of Rajasthan Jaipur, India.

C. tora possess various medicinal and pharmacological activities these includes antihepatotoxic [13], antiallergic [14], antimutagenic [15]., antifungal [16], radical scavenging and antimicrobial [16-25] .The chemical constituent of the plant *C. tora* are rubrofusarin triglucoside, non rubrofusarin gentobioside, demethyl-flavasperone, genitobioside, torachrysone gentibioside, torachrysone tetraglucoside, tora chrysone apioglucoside, torachrysone, toralactone aloemodin, rhein, emodin, naphthalene, anthraquinone, methicillin-resistant [23-26].

Preparation of Test material- *C. tora* plant (RUBL20672) had been identified by the deposit herbarium specimen stem at the Department of Botany, University of Rajasthan Jaipur, India. The fresh fruits of the plant *C. tora* were collected around the Jaipur district in the months of September and October of 2010. Washed, shade dried and crushed to make fine powder for further use. The 50% alcohol extract of fresh fruits of this plant was prepared according to WHO protocol CG-04 [27].

Animal model- Colony-bred, healthy adult fertile male Wistar rats (*Rattus norvegicus*) weighing between 150-200gm about 50-60 days aged were used for the present study. The animals were kept in polypropylene cages, measuring 430×270×150 mm and housed under controlled environment conditions with the provision of 12 hrs light: 12hrs dark conditions. The animals were fed with platted standard rat chow (Ashirwad, Chandigarh), supplemented with soaked gram and wheat, water was provided *ad libitum*. Body weight of each animal in all groups was measured weekly to see the possible changes in body weight throughout the experiment.

Experimental design and Protocol- Rats of similar body weight, size, age were grouped as under. Whole study was divided into two experiments. Following experiments were carried out during the course of study, to observe anti-fertility effect and to observe the mode of action/effects nature of the extract and reversibility effects.

The animals were divided into 5 treatment groups, each consisting of 10 animals

Group-A: The animals were given sterile DW alone orally; serve as vehicle treated controls.

Group-B: The animals of this group were treated with *C. tora* (50% EtOH) at 50 mg/kg.b.wt/day.

Group-C: The animals of this group were treated with *C. tora* (50% EtOH) at 100 mg/kg.b.wt/day.

Group-D: The animals of this group were treated with of *C. tora* (50% EtOH) at 200 mg/kg.b.wt/day.

Group-E: The animals of this group were treated with *C. tora* (50% EtOH) 100 mg/kg. wt/day for 60 days were kept for a recovery period of 30 days.

Body organs weights- The initial final body weights of the animals were recorded. Testes, epididymis, seminal vesicles ventral prostate were dissected out, freed from adherent tissues weighed to the nearest milligram on an electronic balance.

Tissue Biochemistry- The testis, epididymis, seminal vesicles ventral prostate were dissected out, freed from adherent tissues weighted in the nearest milligram balance. Protein [28], glycogen [29], cholesterol [30], sialic acid [31], ascorbic acid [32], fructose [33] were estimated in right side of testis other accessory reproductive organs.

Fertility Test- Male rats were introduced to female, 200–300 gm (male: female ratio, 1:2) at 17:00 h after 55 days of treatment. The mated females were allowed to complete the gestation. The number of pups was recorded litter size percent fertility was calculated [27].

Sperm Motility and Density- For sperm motility density, 50 mg of cauda epididymis was minced in 1 ml of physiological saline, immediately within 5 min after scarification; 1 drop of evenly mixed sample was applied to a glass slide under a cover glass. The percent motility was determined by counting both motile immotile spermatozoa per unit area. After that cauda epididymal sperm density was made by routine procedure express as millions/mm^3 suspension [34].

Hormone Assay- Blood samples were also collected for serum separation to estimate FSH, LH and testosterone by radioimmunoassay. Serum samples separated by standard procedures stored at -20°C for subsequent analysis. Serum levels of testosterone were assayed in duplicate using a radioimmunoassay kit [35].

Histopathological Study- Contra lateral side of the testis, epididymis, vas deferens, seminal vesicle, ventral prostate, kidney, heart, liver were fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in xylene and free from adherent tissue and embedded in paraffin wax (Melting point 55^o–62^oC). Sections were made in 6 µ was stained with Harris's hematoxylin and eosin to observe histopathological changes.

STATISTICAL ANALYSIS

Statistical analysis was based on biological statistics. All the values of body organ weights, biochemical estimations histometery were averaged expressed as Mean±Standard error (S.E.). Data were expressed as mean±S.E. analyze for statistical significance by using student's "t" test. The data considered as significant and highly significant at $p \leq 0.01$ and $p \leq 0.001$, respectively [36].

Ethical Aspects- The study was carried out under the supervision of ethical committee of the Department of Zoology, University of Rajasthan, Jaipur [Vide Letter No.Rs/98/10/7454 dated 19/8/2010] and CPSEA [37] guidelines were followed to maintain the experimental animals.

RESULTS

Changes in blood and serum profile

C. tora extract treatment in rats caused a non significant change in serum serum proteins, Phospholipids, cholesterol, triglyceride, HDL, LDL and VLDL, acid phasphatase and alkaline phasphatase, LDH, SGOT, SGPT level after the treatment at different dose levels as compared to control rats. *C. tora* extract treated rats did not show any remarkable changes in blood and serum biochemistry and no significant alteration in hematological parameter (data not shown).

Effect on body and reproductive organ weight

Oral administration of extract of *C. tora* (fruit) for 60 days, caused no adverse effect on body weight; whereas the weight of the testes and accessory sex organs decreased significantly (P≤0.001) might be due to decreased level of androgens required to maintain the growth and development of reproductive organs. The weight reduction was dose dependent, i.e. high dose 200 mg/kg.b.wt. (Group D) treated group, drastically reduced followed by less in low dose (Group C) 100 mg/kg.b.wt. group. Significant changes were observed in vas deferens, seminal vesicle and ventral prostate (Table 1).

Table 1: Effect on Body and organs weight of rats treated with *C. tora* extract

Treatment	Initial b.wt. (gm)	Final b.wt. (gm)	Testes mg/100gm b.wt.	Epididymides mg/100gm b.wt.	Vas deferens mg/100gm b.wt.	Seminal vesicle mg/100gm b.wt.	Ventral prostate mg/100gm b.wt.
Group A Control	120.00±2.35	156.50±1.67	1405.79±12.58	595.26±10.70	148.26±2.10	492.42±3.31	76.50±1.72
Group B *C. tora* 50 mg /kg.b.wt.	154.50±1.38	163.50±0.76	1399.51±18.63^{ns}	574.76±9.93^{ns}	145.75±2.13^{ns}	481.45±5.58^{ns}	73.28±0.95^{ns}
Group C *C. tora* 100 mg/kg.b.wt	153.00±1.11	167.50 ±0.83	1381.06±5.86^{ns}	574.30±3.88^{ns}	142.83±2.30^{ns}	479.26±7.80^{ns}	72.74±1.17^{ns}
Group D *C. tora* 200mg/kg.b.wt	114.00±2.08	166.50 ±0.76	1272.46±6.16**	472.74±6.26**	119.54±0.95**	427.80±3.02**	63.86±0.67**
Group E *C. tora* 100 mg / kg. b.wt. recovery	112.00±2.00	160.50±1.17	1374.63±9.70^{ns}	581.88±8.36^{ns}	145.08±1.17^{ns}	488.95±5.53^{ns}	75.81±1.17^{ns}

Data exposed as Mean ±S.E, ns = Non-Significant,* Significant (P≤0.01), Highly Significant (P≤0.001)**

Biochemical changes

The ethanolic extract treatment of *C. tora* in rats were shown significantly decreased (P≤0.001) level of protein, sialic acid, fructose, glycogen, cholesterol contents in reproductive organs. In recovery group (Group-E) after 30 days of withdrawal of treatment, it was altered up to non significant level (Table 2).

Table 2: Changes in level of protein, sialic acid, cholesterol, glycogen, ascorbic acid and fructose level in reproductive organs of rats following *C. tora* extract treatment

Treatment	Protein (mg/gm)		Sialic acid (mg/gm)		Cholesterol (mg/gm)	Glycogen (mg/gm)	Ascorbic acid (mg/gm)	Fructose (mg/gm)
	Testes	Cauda	Testes	Cauda	Testes	Testes	Adrenal	Seminal vesicle
Group A Control	226.19±3.41	223.08±3.23	5.20±0.25	5.44±0.41	16.06±0.47	2.17±0.08	2.90±0.22	4.89±0.31
Group B *C. tora* 50 mg/kg.b.wt	221.75±2.69^{ns}	219.53±3.52^{ns}	4.72±0.28^{ns}	4.68±0.28^{ns}	15.62±1.78^{ns}	2.16±0.06^{ns}	2.85±0.18^{ns}	4.84±0.22^{ns}

Group C								
***C. tora* 100 mg / kg.b.wt**	219.53±4.55 ns	216.42±3.63 ns	4.72±0.22 ns	4.60±0.14 ns	15.69±0.48 ns	2.11±0.10 ns	2.81±0.12 ns	4.36±0.23 ns
Group D								
***C. tora* 200 mg/kg.b.wt**	186.65±3.86**	191.98±3.91**	4.08±0.23**	4.00±0.15*	14.12±0.63**	1.62±0.09**	2.43±0.16 ns	3.61±0.26**
Group E recovery								
***C. tora* 100 mg / kg.b.wt**	216.86±4.01 ns	219.09±5.05 ns	5.12±0.22 ns	4.60±0.23 ns	15.69±0.66 ns	2.14±0.08 ns	2.80±0.18 ns	4.81±0.20 ns

Data exposed as Mean ±S.E, ns = non-significant,* Significant (P≤0.01), Highly significant (P≤0.001)**

Effect on Sperm motility and density

Sperms motility and density in cauda epididymdes decreased significantly (P≤0.001) in rats following extract treatment (Group B-D) in comparison to control rats (Group-A). Although, it was recover up to normal level in rats recovery (Group- E) (Fig. 1).

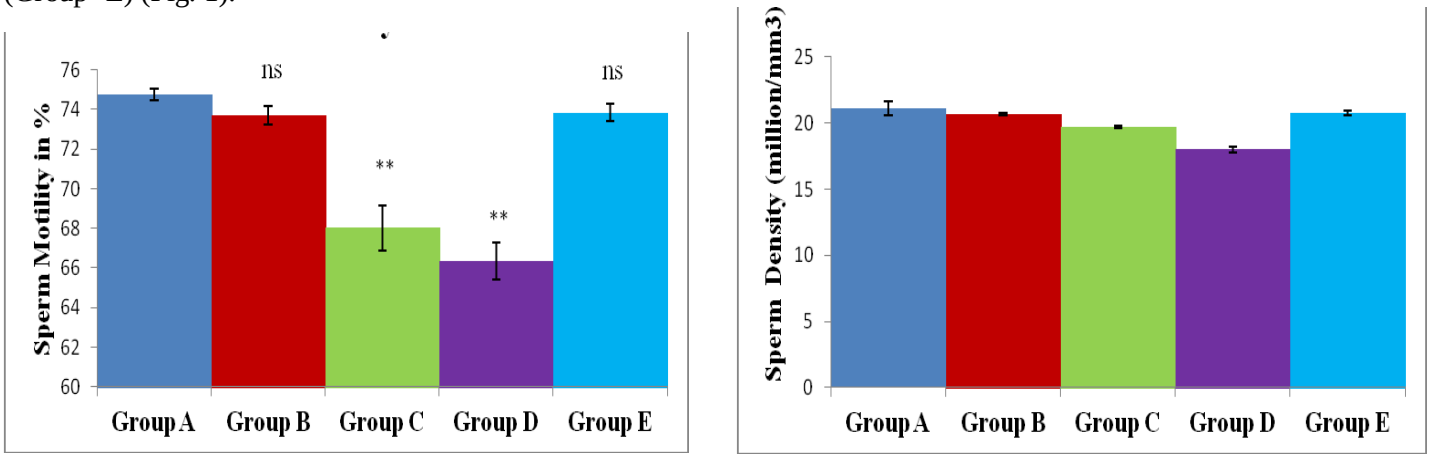


Fig. 1: Effetcts of *C. tora* extract treatment on sperm motility and density

Changes in hormone levels

The level of testosterone, LH and FSH analysis shown the decreased level of these hormones in *C. tora* extracts treated rats (Group B-D) in dose dependent manner at dose levels of 50 mg/ kg b.wt.,100 mg/ kg b.wt.,200 mg/ kg b.wt., however, in recovery group (Group- E) its return up to normal level (Fig. 2-4).

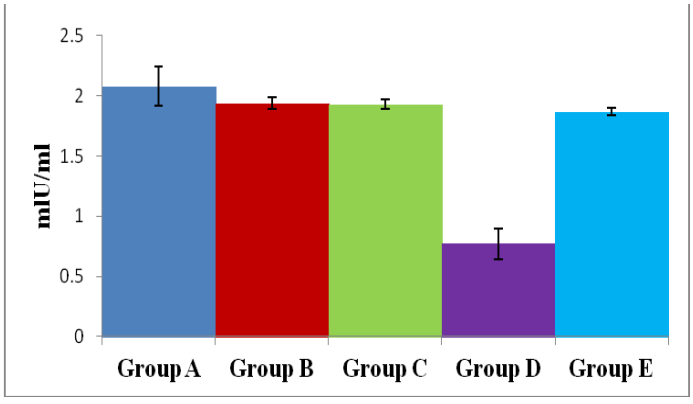


Fig. 2: Effetcts of *C. tora* extract on testosterone level

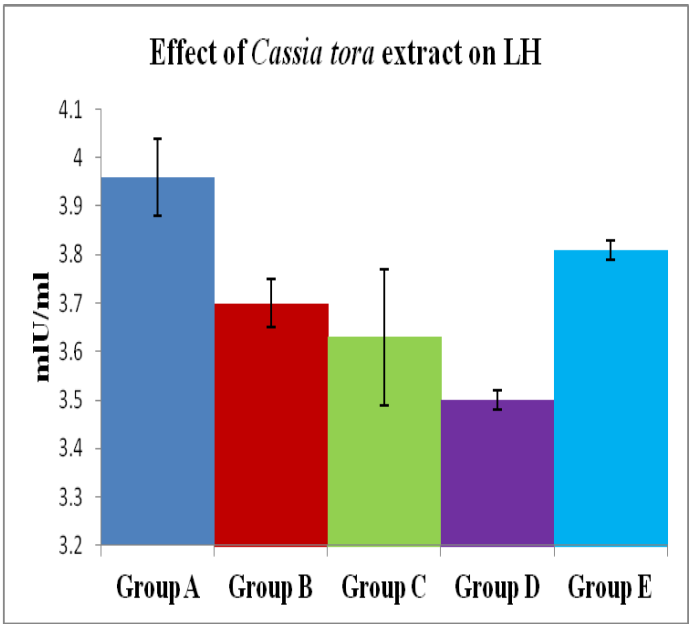


Fig. 3: Effetcts of *C. tora* extract on LH level

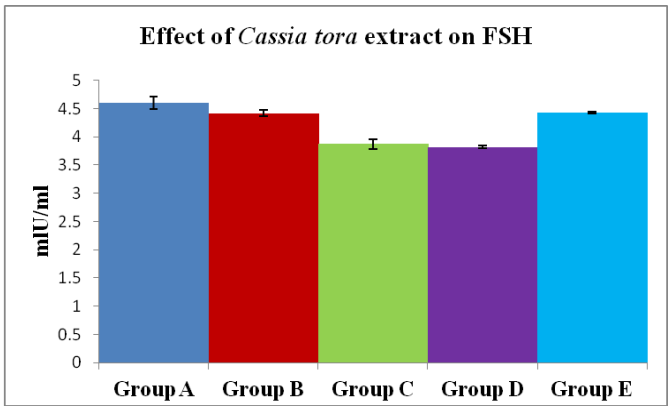
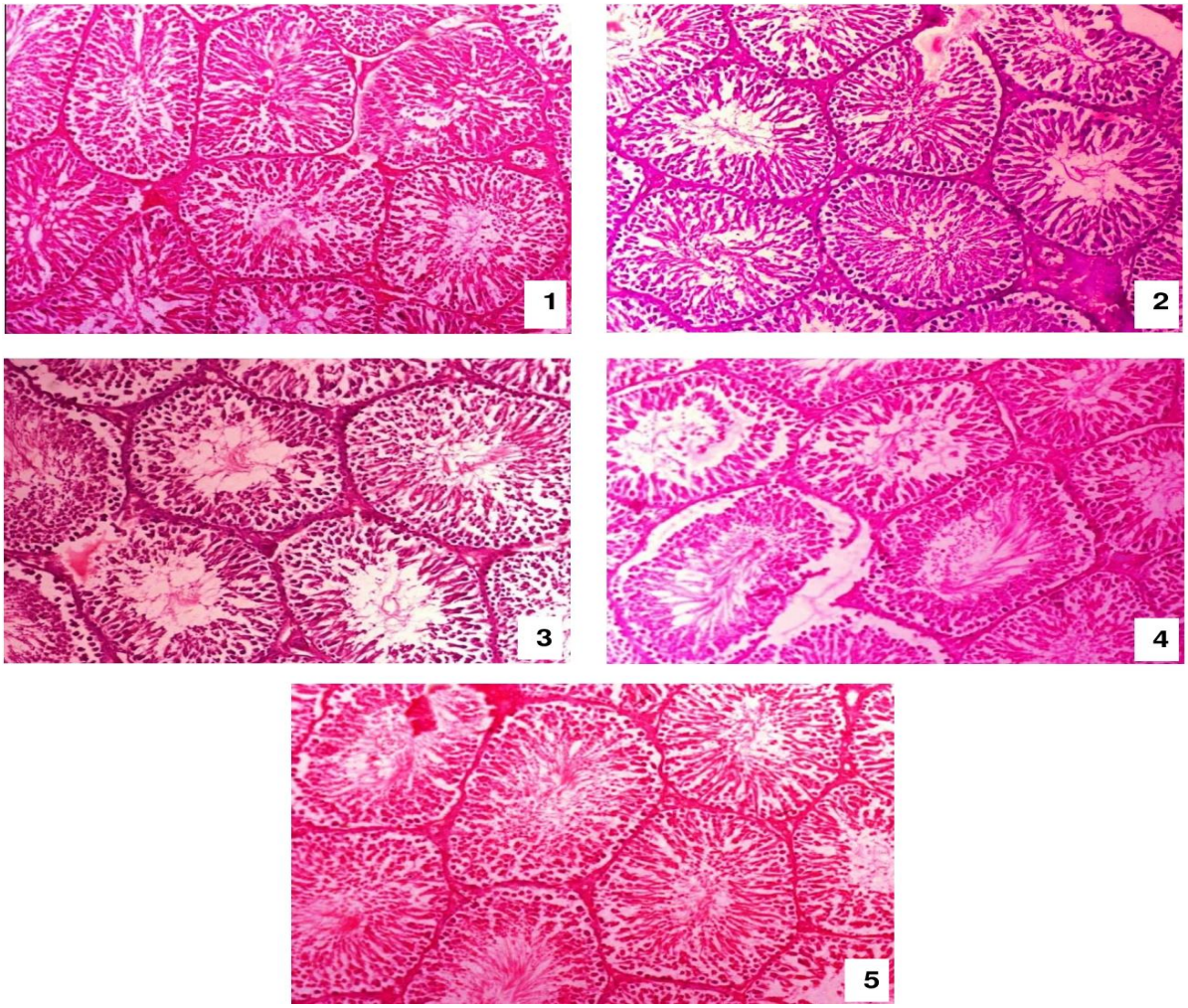


Fig. 4: Effects of *C. tora* extract on FSH Level

Effect on spermatogenesis

Photomicrographs of the testes of *C. tora* treated rats at the dose levels of 50 mg/ kg. b.wt., 100 mg/ kg. b.wt. and 200 mg/ kg. b.wt. caused degenerative changes. Germinal epithelium of seminiferous tubules was ruptured and wavy due to loosened connective tissue. Spermatogonia and sperm number were significantly reduced, cellular debris can be seen in the lumen, inter tubular stroma was increased. However spermatogenesis alters up to normal level in rats of recovery group (Photomicrograph 1-5).

Histopathological Observations of Testes



Photomicrograph-1, Group-A (X 100 H.E.) testis of control rats showing a normal histoarchitecture, well developed germinal epithelium, seminiferous tubules filled with spermatocytes, spermatids and spermatozoa

Photomicrograph-2, Group-B (X 100 H.E.) testes of rat treated at 50 mg/kg b.wt. showing degenerated epithelium, irregular and incomplete spermatogenesis

Photomicrograph-3, Group-C (X 100 H.E.) testes of rat treated at 100 mg/kg b.wt. showing decreased spermatocytes and sperms in seminiferous tubules

Photomicrograph-4, Group-D (X 100 H.E.) testes of rat treated at 200 mg/kg b.wt. showing incomplete spermatogenesis. Primary stages appear to be normal but later stages are absent

Photomicrograph-5, Group-E (X 100 H.E.) testes of rat treated with recovery showing normal semniferous tubule. Spermatocytes, spermatid and spermatozoa are clearly visible in the lumen. Sertoli cell and Leydig cell are also clearly seen

DISCUSSION

Maintenance of structure and functional integrity of accessory reproductive organs requires continuous supply of androgen [8,25, 38-41]. Oral administration of alcoholic extract of *C. tora* (fruit) for 60 days brought a decrease in the weight of reproductive organs indicating that the circulating level of androgen was not enough to maintain the weights of reproductive organs, however the weights of vital organs were not affected. [42-46].

The seminal vesicles are androgen-dependent and this property may be used as a biological marker of androgen activity [47-49]. Protein level is directly correlated with the secretory activity of the epididymis, which in turn depends on the androgen levels [50,51]. The low levels of testicular protein are usually indicative of inhibition of spermatogenesis [52-54]. The decreased sialic acid level in tests and accessory organs of male rats and the epididymis can be a causative factor for impaired sperm function [39,55,56]. A decrease in glycogen content of the testis reduces the energy source for spermatogenic activity which could affect protein synthesis [42,57]. Decrease in ascorbic acid cause hypo functioning of the testis and the degeneration of the germinal epithelium [58]. Fructose serves as source of energy for sperm, reduction in the fructose might be due to the decreased secretory activity of the seminal vesicle [59,60].

Sperm density and motility directly correlates with fertility chances and therefore decreased sperm density [50-54 and motility of spermatozoa might reduced fertility of rats followed extract treatment [61-70]. A large number of metaphasic cells in the germ epithelium of treated animals might be caused by cell cycle blockage or [46,71] arrest at the spermatid level in the form of degenerative changes in the germinal cells together with few fragmented sperms in the lumen and acquired a thick, irregular basement membrane [72-74].

Spermatogenesis requires LH and FSH for initiation and maintenance in male rats. LH, through specific receptors found on the surface of Leydig cells, controls the production and secretion of testosterone. Normal testicular function is dependent on FSH and testosterone is absolutely required for normal spermatogenesis [75-80]. FSH establishes a quantitatively normal Sertoli cell population, whereas androgen initiates and maintains sperm production, thus both hormones co-operate via independent functions to enable maximal spermatogenic output [81-85].

CONCLUSIONS

It can concluded that treatment of *C. tora* fruits extract in male rats reduced levels of protein, fructose, ascorbic acid, glycogen, and sialic acid contents might be responsible to caused degenerative changes in germinal epithelium of seminiferous tubules of testes. The decreased level of testosterone further support anti-androgenic effects of the treatment resulted decreased sperm density and motility reduced fertility of extract treated rats.

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